

MEDICAL PLANT MIXTURES AS ADDITIVES IN FUNCTIONAL BAKERY FOOD PRODUCTION

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Abstract: *In this paper, antioxidant activities of 80% ethanol extracts of mixtures of medicinal herbs, "Vitalplant" (Rhamnus Frangula bark (35%), Mentha x piperita L. leaves (20%), Carum Carvi L. seeds (20%), Petroselinum crispum seeds (25%)) and "Gastroherb" (Origanum Vulgare L. (55%), Coriandrum sativum L. seed (30%), Cynara scolymus leaves (15%)), were tested using 1,1-diphenyl-2-picrylhydrazyl (DPPH)-scavenging, antioxidant activity (AOA), Fe²⁺ ions chelating and iron reduction tests.*

Ethanol extracts of Vitalplant and Gastroherb plant mixtures exhibited antioxidant activities in all the assays. Regarding all the results, it can be concluded that plant mixtures Vitalplant and Gastroherb could be regarded as a potential functional food supplements.

Key words: Medicinal Herbs/Antioxidants/Chelating activity/DPPH/Flavonoids/Antioxidant activity/

INTRODUCTION

Antioxidants can protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxy, hydroxyl, and peroxynitrite radicals. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, finally leading to cellular damage. Oxidative stress has been linked to cancer, aging, atherosclerosis, inflammation, and neurodegenera-

tive diseases such as Parkinson's and Alzheimer's diseases. Therefore, antioxidants occurring in plants may play a significant role in health protection (Finkel, 2000).

Spices and herbs have been added to foods since ancient times, not only as flavouring agents, but also as folk medicine and food preservatives. Spices and herbs and their constituents are generally recognized to be

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safe, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies (Smidt et al, 1999). Being natural foodstuffs, spices and herbs appeal to many consumers who question the safety of synthetic food additives.

Presently, there is an increasing interest, both in the industry and in the scientific research for spices and aromatic herbs because of their strong antioxidant properties. These properties are due to many substances, including some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens, minerals (Calucci et al, 2003). Phenolic substances have shown to be the most responsible for the antioxidant activity of plant materials (Rice-Evans et al, 1996). Prevalent phenolics in parsley (*Petroselinum crispum*) are apigenin glycosides (Justesen et al, 2001). In caraway (*Carum Carvi* L.) the main phenolics are quercetin and kaempferol glycosides, while in coriander (*Coriandrum sativum* L.) apigenin, quercetin 3-glucuronide, isoquercitrin and rutin (Suhaj, 2006). The main identified compounds in oregano (*Origanum Vulgare* L.) are protocatechuic acid and its phenyl glucoside, caffeic acid and rosmarinic acid (Kikuzaki et al, 1989), while in buckthorn bark (*Rhamnus Frangula*) there are anthraquinone glycosides, flavonoids and tannins (Newall et al, 1996). In mint (*Mentha x piperita* L.) the main individual compounds are eriocitrin and rosmarinic acid, they account for 59 to 67% of total phenolics (Areias et al, 2001). The main plant chemicals of artichoke (*Cynara scolymus*) are caffeic acid, chlorogenic acid and cynarin (Zhu et al, 2004).

Regarding the fact that *Rhamnus Frangula*, *Mentha x piperita* L., *Carum Carvi* L., *Petroselinum crispum*, *Origanum Vulgare* L., *Coriandrum sativum* L. and *Cynara scolymus* are medicinal plants that are known to have action on digestive stimulation and that they are rich in plant phenolics, responsible for their antioxidant activity, these plants could be considered as the functional food components.

The objective of this research was to evaluate the antioxidant activities of ethanolic extracts of mixtures of these medicinal herbs

using conventional antioxidant tests (1,1-diphenyl-2-picrylhydrazyl (DPPH[•])-scavenging, antioxidant activity (AOA), Fe²⁺ ions chelating and iron reduction) as well as the total phenolics and flavonoids content.

2. MATERIALS AND METHODS

2.1. Plant materials

The mixtures of medicinal herbs "Vitalplant" (*Rhamnus Frangula* bark (35%), *Mentha x piperita* L. leaves (20%), *Carum Carvi* L. seeds (20%), *Petroselinum crispum* seeds (25%)) and "Gastroherb" (*Origanum Vulgare* L. (55%), *Coriandrum sativum* L. seed (30%), *Cynara scolymus* leaves (15%)), are the products of the Institute for Medicinal Plant Research "Dr Josif Pančić" from Belgrade. The herbal mixtures were in the form of powder, granulation up to 3 mm.

2.2. Preparation of plant extracts

About 10,0 g of each plant mixture was shaken with 100 ml of 80% ethanol, at room temperature for 1 h, and the mixture was left to stand overnight. The residues obtained after filtration were reextracted twice with 80% ethanol by shaking for 1 h and filtrated. The combined extracts were evaporated to dryness in the rotary evaporator at 40 °C, and stored at -4 °C until further use.

2.3. DPPH[•] free radical-scavenging activity test

Plant extracts were tested in DPPH[•] free radical-scavenging activity test. Plant extracts were dissolved in methanol, in appropriate manner to obtain a series of dilutions (0.07-0.80 mg/ml).

Radical-scavenging activity against the stable radical DPPH[•] (1,1-diphenyl-2-picryl-hydrazyl radical (Sigma, USA) was determined spectrophotometrically following the procedure of Espin et al. (Espin et al, 2000). IC₅₀ (mg/ml) was defined as the concentration of an antioxidant extract which was required to quench 50% of the initial DPPH[•] under the given experimental conditions. IC₅₀ was ob-

tained by interpolation from linear regression analysis.

2.4. Antioxidant activity of plant extracts

Antioxidant activity of plant extracts, based on coupled oxidation of β -carotene and linoleic acid was determined according to the method of Moure et al. (Moure et al, 2001). Degradation rate of the extracts was calculated according to first order kinetics, and the antioxidant activity (AOA) was expressed as % inhibition relative to the control (Al-Saikhan et al, 1995). IC_{50} (mg sample/ml) was defined as the concentration of an antioxidant extract which was required to inhibit the degradation of β -carotene 50% relative to the control under the given experimental conditions. IC_{50} was obtained by interpolation from linear regression analysis.

2.5. Total phenolics and total flavonoid content of plant extracts

The content of total phenolics in plant extracts, measured as gallic acid equivalents was determined using Folin-Ciocalteu's reagent (Singleton et al, 1999). The flavonoids content in the extracts was measured by the $AlCl_3$ method based on the formation of a flavonoid-aluminium complex. Rutin was used for calibration curve preparation (Dewanto et al, 2002).

2.6. Reducing power of plant extracts

The reducing power was determined by measuring the formation of Perl's Prussian blue at 700 nm (Oyaizu, 1986). IC_{50} value (mg extract/ml) is the effective concentration at which the absorbance was 0.5 for reducing power, and was obtained by interpolation from linear regression analysis. BHT (butylated hydroxy toluene) was used for comparison.

2.7. Chelating activity on ferrous (Fe^{2+})

Chelating activity of ethanolic extracts on Fe^{2+} ions was measured according to the method of Decker & Welch (Decker et al, 1990), with minor modifications. In our experiment, $FeSO_4$ (2 mM) was used instead of $FeCl_2$. Chelating effects were measured at

three different concentrations, and then plots of chelating effects versus concentration were made and values of IC_{50} were determined as effective concentrations at which Fe^{2+} ions were chelated by 50%.

3. RESULTS AND DISCUSSION

Obtained results for total phenolics content, total flavonoids content, DPPH \cdot scavenging activity test, AOA and reducing power of plant extracts are presented in Table 1.

The yields of ethanolic extracts of Vitalplant and Gastroherb mixtures were 17.3 and 15.7%, respectively. The total phenolic content as well as the total flavonoids content of each extract was estimated, since phenolics and flavonoids may significantly contribute to its overall antioxidant activity. The amount of total phenolics as well as the composition of the extract is highly dependant on extraction method and the type and polarity of extraction solvent (Moller et al, 1999). Higher contents of total phenolics are found in Gastroherb plant mixture, while the content of total flavonoids was found to be higher in Vitalplant mixture.

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity measures hydrogen-donating ability of antioxidants. Activity is measured as the relative decrease in absorbance of DPPH as it reacts with the antioxidant. DPPH free radical-scavenging activity of investigated plant extracts was tested at four different concentrations, and was highly concentration dependent. Both extracts showed linear regression between absorbance (A) and concentration (c) (Vitalplant $A = 55.529 \cdot c - 0.2674$, $R^2 = 0.9999$, Gastroherb $A = 135.16 \cdot c - 0.6892$, $R^2 = 0.999$) within investigated concentration range (0.02-0.80 mg/ml). Obtained IC_{50} values indicate that ethanolic extract of Gastroherb plant mixture possessed better free radical-scavenging activity (Table 1).

IC_{50} values for AOA, reducing power and chelating activity are presented in Table 2. Reducing power is often used as an indicator of electron-donating activity, which is an im-

portant mechanism of phenolic antioxidant mechanism (Yildirim et al, 2001).

Antioxidants reduce the ferric ion/ferricyanide complex to the ferrous form, the Perl's Prussian blue complex. For the concentration range between 0.02 – 0.70 mg/ml, dependence between reducing power and concentration was linear (Vitalplant $A = 0.3352 \cdot c + 0.0287$, $R^2 = 0.9964$, Gastroherb $A = 0.7372 \cdot c + 0.0286$, $R^2 = 0.9992$). Obtained IC_{50} indicate that ethanolic extract of Gastroherb plant mixture possessed better reducing

power (Table 1). IC_{50} for BHT was 0.119 ± 0.007 mg/ml.

AOA was tested in a β -carotene/linoleic acid model system, β -carotene undergoes rapid discoloration in the absence of an antioxidant. Vitalplant mixture exhibited better AOA activity, which is indicated by lower IC_{50} (Table 1). Like for other antioxidant parameters good linearity between concentrations and AOA was achieved (Vitalplant $A = 8.944 \cdot c - 2.8351$, $R^2 = 0.9798$, Gastroherb $A = 9.3582 \cdot c - 8.6634$, $R^2 = 0.9998$), for the concentration range 0.78-70 mg/ml.

Table 1.

Total phenolics content, measured as gallic acid equivalents (% dry herb mixture), total flavonoids content (% dry herb mixture), measured as rutin equivalents and DPPH[•] scavenging activity of ethanolic extracts (mg extract/ml).

| | Total phenolics (% D.M.) | Total flavonoids (% D.M.) | DPPH [•] IC_{50} (mg/ml) |
|------------|-----------------------------|------------------------------|--|
| Vitalplant | 1.14 ± 0.08 | 0.36 ± 0.01 | 0.89 ± 0.02 |
| Gastroherb | 1.55 ± 0.09 | 0.23 ± 0.01 | 0.38 ± 0.04 |

Table 2.

IC_{50} (mg extract/ml) values for AOA activity, reducing power and chelating activity on Fe^{2+}

| | AOA IC_{50} (mg/ml) | Reducin power IC_{50} (mg/ml) | Chelating activity on Fe^{2+} (mg/ml) |
|------------|--------------------------|------------------------------------|--|
| Vitalplant | 5.61 ± 0.54 | 1.27 ± 0.26 | 0.98 ± 0.04 |
| Gastroherb | 6.35 ± 0.09 | 0.60 ± 0.06 | 0.56 ± 0.07 |

For chelating activity, within investigated concentration range of extracts (0.1-1.4 mg/ml), good linearity was achieved in both investigated cases (Vitalplant $A = 48.87 \cdot c + 0.5624$, $R^2 = 0.9967$, Gastroherb $A = 46.185 \cdot c + 20.54$, $R^2 = 0.9738$). Regarding the IC_{50} values, Gastroherb mixture was more efficient. Antioxidants inhibit interaction between metal and lipid through formation of insoluble metal complexes with ferrous ion or generation of steric hindrance.

The iron-chelating capacity test measures the ability of antioxidants to compete with ferrozine in chelating ferrous ion. Activity is measured as the decrease in absorbance of the red Fe^{2+} /ferrozine complex.

4. CONCLUSIONS

Extracts of Vitalplant and Gastroherb plant mixtures exhibited antioxidant activities in all the assays. Both mixtures contained significant quantity of plant phenolics and flavonoids, which might be the most responsible compounds for their antioxidant activity. Gastroherb was more efficient in all assays, with the exception of AOA test. Regarding all the results, both mixtures could be considered as potential functional food components.

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